Abstract.—A gross measure of reproductive condition (ovary weight adjusted for body size and oocyte volume) is developed and evaluated as an alternative to commonly used gonad indices, for classifying the maturity status of individual ehu (Etelis carbunculus) and kalekale (Pristipomoides sieboldii), two species of eteline snappers (Lutjanidae) that contribute to the deep-slope handline fishery in Hawaii. Discriminant analysis and logistic regression, based on body length, ovary weight, and oocyte volume, were used to classify fish as either immature or mature. Discriminant analysis correctly classified the maturity of about 98% of both ehu and kalekale, with histological criteria as the standard for comparison. Logistic regression correctly classified maturity of 97% of the ehu and 100% of the kalekale. Misclassification errors increased by 3.75-5% (discriminant analysis) or 0–5% (logistic regression) if oocyte volume was excluded and only body length and ovary weight were used as predictors of maturity.

For kalekale, estimates of lengths at which 50% are sexually mature  $(L_{50})$ were identical (29.0 ±1.8 [SE] cm fork length, FL;  $r^2=0.92$ ), when maturity was classified histologically or by logistic regression on body, ovary, and oocyte metrics. For ehu,  $L_{\rm 50}$  estimates were similar ( $r^2 \approx 0.96$ ) for maturity assignments based on histology (27.9  $\pm$ 2.4 cm FL) and on logistic regression using gross metrics (27.8  $\pm$ 2.3 cm FL).

We conclude that gross morphometrics can provide adequate proxies for histological evidence when categorizing sexual maturity in asynchronous, multiple-spawning fishes like eteline lutjanids. The potential benefits of using gross metrics for assessing sexual maturity in other serial spawners are briefly discussed.

# Morphometric criteria for estimating sexual maturity in two snappers, Etelis carbunculus and *Pristipomoides sieboldii*

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Body size at sexual maturity is of fundamental importance in fishery stock assessment (e.g. when calculating a "spawning potential ratio" [SPR, Goodyear, 1993; Somerton and Kobayashi<sup>1</sup>]). In fisheries applications, average size at maturity is usually expressed as the body length at which 50 percent of females are mature  $(L_{50})$ , and is typically estimated by fitting a model such as the logistic (Gunderson et al., 1980; O'Brien et al., 1993) to observed percentages of mature fish within several length classes. Estimating percentage mature by size class assumes that individuals can be accurately classified as either immature or mature.

Various methods have been used to classify individual female fish as sexually mature, including gross inspection of ovaries, light microscopic examination and measurement of whole oocytes in fixed tissue samples, histological stains of intraovarian inclusions, and an array of ratios, commonly referred to as "gonad indices" or "gonadosomatic indices" (GSIs) that standardize gonad mass to body size. These methods differ in precision, accuracy, and processing time. Gross inspection of ovaries is quick but highly subjective and often cannot fish (West, 1990). Light microscopy

of whole oocytes can effectively identify the most advanced oocyte mode only if the dynamics of oocyte growth are known for the particular species and may be inaccurate for ovaries that also contain oocytes in intermediate stages of vitellogenesis (West, 1990). Histological staining of tissue samples for detection of cytoplasmic inclusions (yolk, oil globule presence and number) in the most advanced oocytes provides the best evidence of maturation and is the most accurate method, but it is also the most time consuming and expensive (Delahunty and deVlaming, 1980).

Usually only a single method has been used in a given study, although some studies (e.g. Cayre and Laloe, 1986; Hay et al., 1987; Kjesbu, 1991; Olsen and Rulifson, 1992; Ramsay and Witthames, 1996) have at least attempted to confirm maturity classifications with more than one technique. Average size at maturity has usually (e.g. Higham and Nicholson, 1964; DeMartini and Fountain, 1981), but not always (Erickson et al., 1985a; Matsuyama et al., 1987; McQuinn, 1989; Hunter et al., 1992;

<sup>1</sup> Somerton, D. A., and D. R. Kobayashi.

1990. A measure of overfishing and its

application on Hawaiian bottomfishes. Honolulu Laboratory, Southwest Fish. Sci. be used to distinguish between im-Cent., Natl. Mar. Fish. Serv., NOAA, Homature and spent (resting) mature Manuscript accepted 28 August 1998. nolulu, HI 96822-2396. Southwest Fish. Sci. Cent. Admin. Rep. H-90-10, 18 p.

Render et al., 1995), been identified subjectively if more than one method of classification was used, sometimes with inconsistent results (Trippel and Harvey, 1991). Recently, West (1990) identified microscopic staging coupled with a gonad index as the most objective and quantitative method.

Gonad indices should be used with caution as measures of sexual maturity and require biologically realistic companion analyses (e.g. Koya et al., 1995). A fundamental flaw of ratios, such as gonad indices, is that they either lose or obscure meaningful information in the numerator and denominator of the ratio (Garcia-Berthou and Moreno-Amich, 1993). Gonad indices also are questionably applicable for asynchronous, multiple-spawning fishes (deVlaming et al., 1982). There are two major reasons for this. First, because multiple-spawners ripen and shed numerous batches of eggs, the ovary weight of an individual fish fluctuates cyclically and often greatly during the spawning season. Second, somatic weight often varies independently of ovary weight for individuals. Somatic "condition" (robustness) usually declines monotonically, and sometimes greatly, as somatic energy reserves become progressively depleted over the spawning season, whereas ovary weights cycle high and low as the individual continues to spawn (Delahunty and deVlaming, 1980; deVlaming et al., 1982). Variances in GSIs among mature fish are unavoidably large (e.g. Macer, 1974) because small, ready-to-spawn fish sometimes have higher GSIs than larger fish that have just shed their eggs. Other potential complications include differences in ovarian cycling frequencies (spawning intervals) between small and large females within populations (Hunter and Macewicz, 1985). Thus even refinements of the conventional GSI such as the "relative gonadal index" (RGI) of Erickson et al. (1985b), in which gonadal investment is adjusted for size allometry, can be inappropriate for asynchronous, multiple-spawners if the dynamics of somatic and gonadal condition are unknown. Statistical descriptors of sexual maturity that are more accurate and robust than GSIs need to be developed (Garcia-Berthou and Moreno-Amich, 1993; Winters and Wheeler, 1996; Jons and Miranda, 1997).

Our study has several complementary objectives. First, we evaluate four gross metrics (fish body length and weight, ovary weight, and oocyte size) for describing stages of sexual maturation in two eteline lutjanids (ehu, *Etelis carbunculus*, and kalekale, *Pristipomoides sieboldii*) that contribute to the deepslope bottom fishery in Hawaii. We examine these four characters because they are readily measured and less costly than the more accurate histological staining of ovarian tissue. We also independently

assess stage of sexual maturity for each species based on histology of ovarian tissue samples from specimens collected during peak periods of spawning. In our study, we use discriminant analysis and logistic regression to further classify individual fish as either immature or mature on the basis of gross morphometric and histological criteria. In conclusion, we evaluate the results of gross classifications of maturity for ehu and kalekale by assessing how well  $L_{50}$  estimates based on statistical assignments of maturity with gross metrics agree with  $L_{50}$  estimates based on histological evidence of maturity.

## Materials and methods

## Fish collection and shipboard processing

Two types of fish collections were used: hook and line and bottom trawling. For exploitable-sized fish of both species, hydraulic handline gurdies with sizes 28 and 34 Izuo circle hooks (baited with squid strips) were used to fish near bottom at depths of 60-300 m, on insular slopes and submerged banks located between 22-25°N lat. and 160-170°W long. in the Northwestern Hawaiian Islands (NWHI). Specimens were collected in September 1992; during June, July, August, and September 1993; in August 1996, and in July and August 1997 (Table 1). Most line-fishing was conducted during daylight hours (0730–1930) aboard the NOAA ship Townsend Cromwell. Additional small kalekale were sampled at 60-65 m off south Molokai, Main Hawaiian Islands, during August 1992 and September 1993. We also examined additional small ehu caught by bottom trawl at 88-97 m near Kure Atoll (NWHI) in early June 1977 (Table 1). All specimens examined were collected during the protracted summer spawning period of eteline snappers in Hawaiian waters (Ralston, 1981; Everson, 1984; Everson et al., 1988). Ehu are thought to be serial spawners (Everson, 1984), and oocyte size distributions within ovaries suggest that Hawaiian populations of both species comprise asynchronous spawners.<sup>2</sup>

Once captured, fish were placed immediately on ice. Within an hour of capture, fork length (FL) to the nearest 0.1 cm and ovary-free body weight (OFBW, in g) was estimated for each fish.

Ovary weight (OW) of each specimen that appeared female was estimated  $\pm 0.1$  g (if <5g),  $\pm 1$  g (if 5–100 g) and  $\pm 10$  g (if >100 g). A tissue plug (1–50 g, de-

<sup>&</sup>lt;sup>2</sup> 1994. Honolulu Laboratory, Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Unpubl. data

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Summary statistics for ehu (*Etelis carbunculus*) and kalekale (*Pristipomoides sieboldii*) used in analyses. Months were sampled in the years 1992, 1993, 1996, and 1997. FL = fork length; OFBW = ovary-free body weight; OW = ovary weight.

		FL (mm)		OF	BW (g)			
Species	Month	Median	Min-Max	Median	Min-Max	Median	Min-Max	n
Ehu	Jun-Jul	368	91-551	865	15-2984	10	0.1-136	37
	Aug	356	203-544	826	200-3260	24	0.3 - 350	80
	Sep	370	270-595	894	351-3615	28	3.0 - 200	64
	Total	362	91-595	850	15-3615	23	0.1 - 350	181
Kalekale	Jun-Jul	340	334-390	714	596-1054	40	26-46	5
	Aug	340	156-438	626	51-1400	20	0.1 - 120	51
	Sep	363	150-412	846	51-1247	17	0.1 - 39	24
	Total	348	150-438	710	51-1400	20	0.1-120	80

pending on ovary size, and including the ovarian wall) was taken from the central one-third of either the right or left ovary (random choice) and placed in 10% formalin sea-water.

## Laboratory processing

Most ovary specimens were processed within 1–2 months after collection. Diameters of 25 of the largest viable oocytes were then measured (random axis, 63× magnification) for each specimen with a dissecting microscope. The median of 25 diameters provides a cost-efficient estimator of average maximum oocyte size for ehu (Lau and DeMartini, 1994).

For each preserved ovary specimen, a subsample of the ovary was dehydrated, imbedded in paraffin, and a minimum of three sections was cut (at 6 µm) and stained with Harris's hematoxylin, followed by eosin counterstain (Hunter and Macewicz, 1985). Slide sections were examined with a compound microscope at 40-500× for the presence and relative quantity of eosinophilic yolk (unyolked oocytes: class 1 of Murphy and Taylor, 1990; partly yolked: classes 2-3; yolked: classes ≥4). If oocytes were yolked, we further noted the presence of postovulatory follicles (POF: class 6), hydrating or hydrated oocytes (HYD: class 7), and oocytes undergoing  $\alpha$  or  $\beta$  atresia (class 8: Murphy and Taylor, 1990). Individuals were designated as "immature" if the most advanced oocytes were unvolked or partly volked without substantial atresia, "ripening mature" if fully yolked but lacking POF or HYD, "resting mature" if a majority of the yolked oocytes were atretic, or "ripe mature" if POFs or HYDs were present. Fish that might in the future have resorbed yolked oocytes without spawning were necessarily classified as mature.

## Statistical analyses

We used oocyte volume (OV) to characterize egg size because a volumetric measure was more directly proportional to OW than was a linear measure such as oocyte diameter. OV was calculated as the volume of a sphere,  $4/3 \pi r^3$ , where r was one-half of the median diameter of the largest oocytes (Lau and DeMartini, 1994). The sampling distributions of dependent and regressor variables were first examined by using raw and log-transformed data. Log-transformed data were least skewed; logarithms (base 10) were therefore used in all subsequent analyses.

We used analysis of covariance (ANCOVA) to determine if OW was suitable for quantifying maturation state of individual fish after adjusting OW for body size, egg size, or both measures. For each species, the analysis evaluated fish of three ovarian developmental stages (unripe immature, ripening or resting mature, and ripe mature), identified histologically. We were thereby able to evaluate the potentially confounding effects of oocyte development and body size on ovary weight (Erickson et al., 1985b).

We further used two classification techniques (discriminant analysis and logistic regression) to distinguish between mature and immature fish. These two techniques were used because we recognized the general need, in studies such as this, to classify the maturity status of individual fish 1) based on both gross morphometrics and histological criteria and 2) with gross metrics alone, without the added benefit of histological evidence. At a minimum, ovary tissues of some of the fish examined in any study need to be examined histologically as a check on the accuracy of gross classification.

Both logistic regression and discriminant analysis can be used to classify dichotomous states such

as maturity. The latter is often (Efron, 1975), but not always (Prager and Fabrizio, 1990), the more effective technique. However, it is less robust to violations of the assumption of identical covariance matrices. Multivariate normality is also assumed if linear discriminant functions are fitted parametrically (Press and Wilson, 1978), an assumption we avoided in this study (see below).

For combined gross-histological classifications of maturity, we used predictive discriminant analysis (Huberty, 1994) to distinguish between mature (ripe, ripening or resting) and immature fish. Nonparametric discriminant functions were derived by using the uniform-kernel method with Euclidean distances (Huberty, 1994). We used logistic regression (Hosmer and Lemeshow, 1989) to evaluate whether maturity classifications based on histology could be predicted by the relationships among body size, OW, and OV. Logistic regression coefficients were estimated by maximum likelihood methods. Using each technique, we evaluated the full (three-variable) model first, then a two-variable model without OV. Because sample sizes were small for each species, all sample fish of each species were evaluated histologically.

Once all individuals had been assigned to maturity classes based on histological and gross morphometric criteria, we assigned specimens to 2-cm length classes and calculated the percentage mature for each length class using each set of criteria. Percent maturity (0–100%) was then related to length class by using nonlinear regression weighted by the square root of the numbers of fish in each length class. The logistic model,

$$P_X = 100 / (1 + e^{-(a+bFL)}),$$

where  $P_x$  = percentage mature in each length class; a and b = fitted parameters; and

 $L_{50} = (-a/b)$ , was fitted to the data by using maximum likelihood.

Data were analyzed using STATISTIX (v. 4.1; Analytical Software, 1994: logistic regression) and SAS (v. 6.03; SAS Institute, Inc., 1988: PROC REG, GLM, and DISCRIM).

## Results

## **Body size interrelations**

After log-transformation, the influences of body length (FL) and ovary-free body weight (OFBW) on ovary weight (OW) were virtually identical; hence, FL (the easier variable to measure) only is reported

in Table 2 and used in all subsequent analyses. OFBW was significantly related to FL for each species (ehu:  $\log OFBW=-1.720 + 2.971\log FL$ ,  $r^2=0.979$ , n=172, P<0.001; kalekale:  $\log OFBW=-1.785 + 3.010\log FL$ ,  $r^2=0.966$ , n=75, P<0.001).

# Effect of ovarian stage on relation of ovary weight to body length

The OW-to-body length relation marginally differed with ovarian developmental stage for ehu and kalekale (FL  $\times$  stage interaction in ANCOVA:  $F_{2,175}$ =3.19 and  $F_{2,74}$ =2.69, P=0.04 and 0.06, respectively; reject  $H_0$ : slopes equal). Thus, ovary weights could not be adjusted by body lengths and subsequently used to distinguish among maturation states for either species. Body length was a poor predictor of ovary weight for both ehu and kalekale ( $r^2$ =0.58 and 0.60, respectively; Table 2).

## Effect of egg size

FL and OV together, however, accounted for 91% (ehu) and 82.5% (kalekale) of the variation in OW (Table 2). Colinearity between FL and OV (or OFBW and OV) was unimportant. For example, FL explained only 12% of the variation in OV for ehu.

## Histological evidence of maturity

Most specimens of ehu and kalekale were readily classified as either immature or mature according to histological criteria. Kalekale included fish that were immature, ripening mature, and ripe mature (none were resting mature). Unlike kalekale, a minority of the large (>40 cm FL) ehu were resting mature, with ovaries undergoing substantial atresia. The ovaries of kalekale usually contained HYD oocytes if POFs were present (19 of 21 cases). Ehu included specimens with HYD oocytes (7 cases) or POFs (98 cases), but POFs were not observed if HYDs were present (0 of 7 cases). Ehu and kalekale clearly differed in the co-occurrence of HYD oocytes and POFs (Fisher exact test; *P*<0.001), suggesting that the average spawning intervals of individual females probably differ between these two species.

## Statistical classifications of maturity

For both species, maturity classifications based on discriminant analysis of the three gross metrics (body length, ovary weight, and oocyte volume) generally agreed (≈98% correct) with those where histological criteria were used (Table 3). Maturity classes determined by histology also were accurately predicted

Table 2

Multiple regression models describing the interrelations of ovary weight (OW), fork length (FL), and oocyte volume (OV) for two species of eteline lutjanids in Hawaii (ehu, *Etelis carbunculus*; kalekale, *Pristipomoides sieboldii*). Mean square errors (MSE) and standard errors of estimates (SE) are noted, with coefficients and standard errors (SE) of model parameters expressed as base 10 logarithms. All regressions are significant, as are the intercepts (all *P*<0.001).

	Model					Coefficie	nt		SE		
Species	$Y = f(X_1 + X_2 + X_1 X_2)$	$R^2$	n	MSE	$b_1$	$b_2$	$b_{12}$	$SE_{b1}$	$SE_{b2}$	$SE_{b12}$	Intercept
Ehu	OW = f(FL)	0.579	172	0.102	4.541	_	_	0.297	_	_	-5.839
	OW = f(FL + OV)	0.911	172	0.022	3.284	0.457	_	0.146	0.018	_	-5.508
	$OW = f(FL + OV + FL \times OV)$	0.917	172	0.020	1.077	501	0.627	0.646	0.274	0.179	-2.149
Kalekale	OW = f(FL)	0.596	75	0.111	7.007	_	_	0.676		_	-9.579
	OW = f(FL + OV)	0.825	75	0.049	3.877	0.376	_	0.551	0.039	_	-6.297
	$OW = f(FL + OV + FL \times OV)$	0.826	75	0.049	4.424	0.583	139	2.308	0.849	0.569	-7.108

### Table 3

Summary results of discriminant analysis evaluating the relations among fork length (FL), ovary weight (OW), plus oocyte volume (OV), for ehu (*Etelis carbunculus*) and kalekale (*Pristipomoides sieboldii*). Analogous summary results using FL and OW only. Imm = immature; Mat = mature.

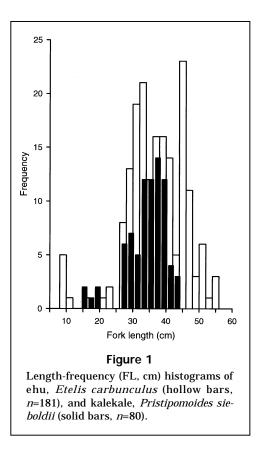
	No.	No.			
Species	classified by histology as	Imm	Mat	Total	% Error
FL, OW, plu	s OV				
Ehu	Imm	33	0	33	0.00
	Mat	3	145	148	2.03
	Total	36	145	181	1.66
Kalekale	Imm	15	0	15	0.00
	Mat	1	64	65	1.54
	Total	16	64	80	1.25
FL and OW					
Ehu	Imm	33	0	33	0.00
	Mat	12	136	148	8.11
	Total	45	136	181	6.63
Kalekale	Imm	14	1	15	6.67
	Mat	3	62	65	4.62
	Total	17	63	80	5.00

(ehu: 97%, kalekale: 100%) by logistic regression using FL, OW, and OV as regressor variables (Table 4). Misclassification errors increased by 3.75-5% (discriminant analysis; Table 3) or 0-5% (logistic regression; Table 4) if oocyte volume was excluded as a predictor of maturity.

#### Table 4

Summary statistics for logistic regressions used to classify maturity (immature, mature) of ehu (*Etelis carbunculus*, n=181) and kalekale (*Pristipomoides sieboldii*; n=80). Histological maturity was regressed on log-transformations of three gross metrics (fork length, FL; ovary weight, OW; plus egg volume, OV), and FL and OW only. Intercepts and higher-order terms were tested for each model and species, but are unspecified if insignificant.

		Coe	Coefficient			
Species	Regressor	Estimate	SE	Prob		
Three-varial	ole model					
Ehu	FL			ns		
	OW	-13.10	6.82	0.055		
	OV	-2.64	0.76	0.001		
	$OW \times OV$	8.03	2.58	0.002		
Total df: 1	78					
Overall pe	rcentage error: 2	.8%				
Kalekale	FL			ns		
	OW	-309.4	125.3	0.014		
	OV	-9.55	5.26	0.069		
	$OW \times OV$	125.2	47.2	0.008		
Total df: 7	7					
Overall pe	rcentage error: 0	%				
Two-variabl	e model					
Ehu	FL	-6.08	1.46	< 0.001		
	OW	12.29	2.75	< 0.001		
Total df: 1	79					
Overall pe	rcentage error: 2	.8%				
Kalekale	FL	-1.74	0.61	0.004		
	OW	5.64	1.37	< 0.001		
Total df: 7	8					
Overall pe	rcentage error: 5	.0%				



## Size at sexual maturity

For each species, the length-frequency distribution comprised relatively few individuals <30 cm FL (Fig. 1). Hence, our size-at-maturity characterizations might have been affected by the scarcity of immature specimens.

**Ehu** On average, ehu in Hawaiian waters mature at an estimated 27.9 cm FL (95% CI=25.5–30.3 cm) according to histological criteria or 27.8 (25.5–30.1) cm FL based on logistic regression according to gross criteria ( $r^2$ =0.967 and 0.962, respectively; Fig. 2). The precision of these  $L_{50}$  estimates is reasonable despite the discontinuous lower tail of the size-frequency distribution. Our  $L_{50}$  estimates for ehu provide an adequate objective basis for evaluating gross and histological classifications; however, they should be considered only as preliminary estimates of size at maturity.

Kalekale A body length-maturity pattern also was strongly evident for this species. Whether based on histological evidence or logistic regression with gross metrics, size at maturity was well described by the logistic model ( $r^2$ =0.920; Fig. 3). Kalekale in Hawaiian waters mature at 29.0 (95% CI=27.2–30.8) cm

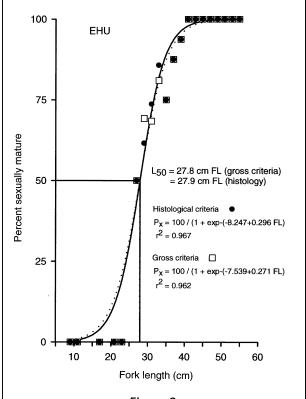


Figure 2

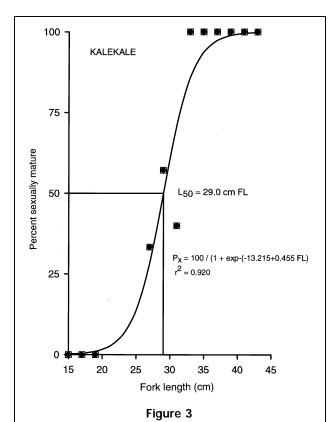
Scatterplots of mean percentage maturity by 2-cm length class, for ehu, *Etelis carbunculus*. Solid circles and solid line represent the observed values and fitted curve based on histological criteria of maturity; hollow squares and dotted line represent the values and curve based on maturity classified by logistic regression on fork length, ovary weight, and oocyte volume. The logistic curves were fitted by nonlinear regression with each class mean weighted by the square root of its count (see Fig. 1).

FL, according to histological and gross criteria (Fig. 3).

## Discussion

## Gross metric criteria

For both ehu and kalekale, the proportional weight of ovaries increased allometrically with body length (coefficients of  $b_1 >> 3$ ; Table 2), which is typical for asynchronous multiple-spawners (Reiss, 1987). Our results further confirm that gonad indices are unsuitable for identifying maturity stages in asynchronous multiple-spawners because the rate at which ovary weight increases with body size differs among fish of different maturation stages (deVlaming et al., 1982; Erickson et al. 1985b). Body size, ovary size,



Scatterplots of mean percentage maturity by 2-cm length class, for kalekale, *Pristipomoides sieboldii*. Solid circles inside hollow squares and solid line represent the congruent observed values and fitted curve based on histological criteria of maturity and on maturity classified by logistic regression on fork length, ovary weight, and oocyte volume. The logistic curve was fitted by nonlinear regression with each class mean weighted by the square root of its count (see Fig. 1).

and oocyte size can be used to classify maturity directly by using either discriminant analysis or logistic regression, however. Logistic regression may be more applicable in most studies because of its less stringent assumptions (Press and Wilson, 1978). We recommend using body length for these types of analyses because length data are easier to obtain than weight data.

## Relative merits of gross and histological evidence

Histological inspection of stained ovary sections provides the most accurate assessment of sexual maturity, but at the greatest cost. Gross morphometrics can be used to characterize maturity at a greatly reduced cost, but are less accurate predictors. The benefit of a gross morphometric proxy depends on whether the loss in accuracy is outweighed by the cost savings. In our study, assignments of sexual

maturity based on discriminant analysis or logistic regression with body length or weight, ovary weight, and oocyte volume were accurate (97–100% correct classification) for ehu and kalekale. Analogous maturity assignments based on only body size and ovary weight (omitting oocyte volume) were less accurate (93–97% correct), indicating a small but real contribution of the egg size variable. Whether the benefit of including an egg size variable merits the cost of its measurement will vary with the particular study. The egg size variable likely had no effect in the logistic regression model for ehu because our specimens included resting mature fish (undergoing oocyte atresia) with small viable oocytes. Two of the three ehu (32.5, 36.2 cm FL) that were misclassified as immature by discriminant analysis with body length, ovary weight, and egg size were resting mature fish. Our ehu data perhaps illustrate discrepancies between gross and histological classifications that might arise when histological criteria for maturity include evidence such as atresia that is unrelated to oocyte mass. Gross criteria likely are most accurate in cases in which samples are collected during peak spawning periods when most fish are actively spawning. Histological criteria may be less than 100% accurate for cases (such as ehu, this study) in which some adolescent fish may resorb yolked oocytes before first spawning (De Veen, 1970; Ramsay and Witthames, 1996). The ability to determine maturity status with reasonable accuracy with only gross metrics in this study nonetheless suggests that such methods warrant further application and evaluation.

## Size at sexual maturity

Ehu Our current best estimate of  $L_{50}$  for ehu in Hawaiian waters ( $\simeq$ 28 cm FL) must be considered preliminary because data for small immature fish are relatively few. Everson (1984), in his pilot evaluation of size at maturity for ehu from Hawaiian waters, also had few small specimens: only 2/185 fish collected during the May–September spawning seasons of 1977–81 were <30 cm FL. Prior to the estimates provided in the present study, those provided by Everson (1984) were the only size-at-maturity estimates available for use in managing ehu in Hawaii (Kobayashi³).

Our paucity of specimens <25 cm FL might have reflected hook-size selectivity (Ralston, 1990). How-

<sup>&</sup>lt;sup>3</sup> Kobayashi, D. R. 1993. Effects of increasing the minimum size limit or imposing fishing closures on three species of Hawaiian deepwater snappers. Honolulu Laboratory, Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Admin. Rep. H-93-01, 18 p.

ever, ehu have relatively large mouths, take baited hooks aggressively (Ralston, 1990), and smallmouthed kalekale were well-represented in our catches. We therefore should have caught small, immature ehu had they been abundant where we fished. Alternatively, juvenile ehu might occupy different bottom depths, as evident for several species of eteline snappers elsewhere (Ralston and Williams, 1988; Parrish, 1989). If so, juvenile ehu inhabit considerably different depths than do larger fish because we fished at depths that were much shallower and deeper than the typical (175–225 m) depths at which adult ehu occur in Hawaiian waters. It is more likely that juvenile ehu occur in different, perhaps lowerrelief microhabitats at or near depths at which larger conspecifics are fished in Hawaii (Clarke, 1972; Struhsaker, 1973).

Our estimate of  $L_{50}$  for ehu approximates the norm for deepwater lutjanids: 28 cm FL is equivalent to 43% of its reported maximum body length in Hawaiian waters of 65 cm FL (Everson, 1984). On average, deepwater insular snappers mature at about 50% maximum body length, although values can range considerably lower and higher among species (Grimes, 1987).

Researchers must not apply size-at-maturity estimates to other populations of the same species for which somatic condition (Ralston, 1988) or size-specific gonadal allocation might differ, because to do so could result in erroneous estimates of size at maturity. For example, body size at maturity estimated for ehu in this report is applicable only to fish in the Hawaiian archipelago. It is almost certain that body size at sexual maturity differs among Pacific regions, given the great geographic variations in average and maximum body sizes and growth rates of ehu (Smith, 1992; Moffitt, 1993). The  $L_{50}$  value for ehu in the South Pacific (Vanuatu), where the species attains maximum body lengths of 120 cm FL, is likely greater than that for ehu in Hawaii. Conversely, our value may be an overestimate in the Marianas, where ehu apparently attain maximum body lengths of less than half those at Vanuatu (Smith and Kostlan, 1991). Improved estimates of  $L_{50}$ , based on more data for smaller fish, are needed for ehu in Hawaii. Better age and growth estimates also are needed for ehu in Hawaii and elsewhere because of uncertainties in present size-at-age and growth-rate estimates for the species (Smith and Kostlan, 1991; Kobayashi<sup>3</sup>).

Kalekale We provide the first direct estimates of size at maturity of this species. In age and growth studies of kalekale from the Marianas (Ralston and Williams, 1988) and Hawaiian Islands (Williams and Smith, 1997), deceleration in somatic growth (which

should reflect gonadal maturation) occurred at lengths of 25–30 cm FL, consistent with our estimated size at maturity for this species. An  $L_{50}$  of 29 cm FL is about 53% of its estimated maximum length in Hawaii (55 cm, as 105% of  $L_{\infty}$ =52.5 cm FL; Fig. 4 in Williams and Smith, 1997), also approximating the 50% norm for deepwater insular lutjanids (Grimes, 1987).

## Conclusions

We believe that gross morphometric measures, such as body size, ovary weight, and egg size, can provide adequate proxies for microscopic histological evidence of sexual maturity in asynchronous multiple-spawners like eteline lutjanids in Hawaii. Applicability may vary among species and may relate to the reproductive dynamics of females, both individually and collectively, within spawning populations. We further suggest that these types of measures can be realistically combined to assess sexual maturity using logistic regression or discriminant analysis for other species of temperate and tropical marine fishes.

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